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EFFECT OF CONSECUTIVE COOLING AND IMMOBILIZATION ON
CATECHOLAMINE METABOLISM IN RAT TISSUES

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16. Abstract The combined effect of two stressor stimuli--cooling and immobilization -- acting successively on the sympathetic-adrenaline system was studied experimentally in rats that were cooled for 8 hours at -7.0°C on the first day and immobilized for 6 hours on the next day. The biochemical and histochemical methods used and the experimental technique involved are described in detail; the results (presented in 4 photomicrographs and 4 tables) are formulated in following conclusions: 1) the successive action of cooling and immobilization results in a stronger decrease in the adrenaline and noradrenaline content in the adrenal gland than that which could be due to a simple summation of the cooling and immobilization effects; 2) successive cooling and immobilization are followed by activation of catecholamine synthesis in the adrenal gland; 3) l-DOPA administration (45 mg/kg 3 times in 2 days) intraabdominally activated catecholamine synthesis in the adrenal glands in both the control and test animals. Therefore, it is concluded that l-DOPA administration can prevent stress-induced changes in the catecholamine content in the adrenal gland.					
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EFFECT OF CONSECUTIVE COOLING AND IMMOBILIZATION ON
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SUMMARY

Metabolism of catecholamines was studied in tissues of rats subjected to cooling for 8 hours at -7° and immobilization for 6 hours on the following day. It was shown that under the consecutive effect of cooling and immobilization a decrease in adrenaline and noradrenaline is noted in the adrenal glands. At the same time, not a simple summation of changes is noted, caused by the individual effect of cooling and immobilization, but there are rather more profound changes. Study of the synthetic properties of the adrenal glands in the presence of the substrate l-tyrosine revealed that upon the consecutive effect of cooling and immobilization, activation of catecholamine synthesis is observed. Administration of l-DOPA (three times in 48 hours at 45 mg/kg intraperitoneally) induced activation of catecholamine synthesis in the adrenal glands of control and test animals. Consequently, administration of l-DOPA prevented changes in the catecholamine content of the adrenal glands, induced by stress.

While changes in the sympathetic-adrenaline system arising under the influence of individual stress stimuli have been studied in great detail in the literature^{2,4}, their consecutive and summary influence on it have been studied to a significantly lesser degree. To study the influence of the consecutive effect of two stress stimuli, cooling and immobilization were chosen, the effect of which has been sufficiently thoroughly studied (4, 10-12, and others). The following were studied in experiments on rats: a. change in content of catecholamines and DOPA in rats subjected to the effects of cold on the first day and immobilization on the second; b. ability to synthesize catecholamines in adrenal gland and heart tissue in vitro; c. influence of in vivo administration of l-DOPA, the precursor of catecholamines, on the content of catecholamines and DOPA in the adrenal glands.

The studies were carried out using biochemical and histochemical methods.

PROCEDURE

In the experiments, non-linear male rats weighing 150-200 grams were used. During the first day of the experiment, the rats were cooled for 8 hours at -7° . On the following day, they were subjected to immobilization for 6 hours at a special stand with clamping of the body and extremities. The rats in the test group were given an l-DOPA solution of 1 ml each (45 mg/kg per dose) intraperitoneally on the first day

of the experiment before the beginning of the cold treatment and twice on the second day: before immobilization and 1.5 hours before the end of the treatment. A corresponding volume of physiologic salt solution was given to the control group during the same period. Immediately after the end of the animals' immobilization, they were decapitated and the adrenaline, noradrenaline, and DOPA contents of the adrenal glands, heart, and hypothalamus were studied. In addition, two groups of rats were studied, the first of which was examined immediately after a 6-hour immobilization (without preliminary cooling), and the second group -- 24 hours after an 8-hour cooling. Physiologic saline solution was administered to both groups of rats, just as in the previous series.

To study the synthetic capabilities of the adrenal glands and heart tissue, crushed organs were incubated at 37 in a Krebs-Ringer bicarbonate buffer (pH-7.4) containing glucose in the presence of a solution containing 1 mg tyrosine (0.4 ml). The final volume of the sample was 2 ml. After 2 hours the reaction was stopped by adding 3 ml 0.1 N HClO_4 solution. In the control sample, 3 ml 0.1 N HClO_4 solution was added before incubation, followed by 0.4 ml l-tyrosine solution. The test and control samples contained either one adrenal gland from one animal or half by weight of the rats heart. Determination of the catecholamine and DOPA content in the experiments was carried out by the fluorometric method⁷. For the histochemical study of the catecholamines, the aqueous

formaldehyde method was used⁹. For the study, the adrenal glands, heart, and carotid arteries were removed. Pieces of tissue were incubated in formalin over ice, then they were frozen and cut in a cryostat at -15° . The slices obtained were enclosed in vaseline and heated at 80° . An M -2 microscope was used for examination and photographing of the specimens. The reliability of changes in absolute amounts of catecholamines was evaluated by Student's method, while the correlative relationship was determined by Spearman's criteria.

RESULTS OF THE STUDY

Table 1 shows test results for the study of the consecutive influence of cooling and immobilization on the content of catecholamines and their precursor DOPA in rat tissues. It was shown that cooling and immobilization induce a decrease in adrenaline, noradrenaline, and DOPA content in the adrenal glands. In the heart, hypothalamus, and blood, no significant shifts were found (table 2).

In the control series of experiments for the study of the individual effect of cold and immobilization, it was shown that under the influence of immobilization alone, a decrease in adrenaline and DOPA content and an increase in noradrenaline is noticed. Twenty four hours after cooling without subsequent immobilization, no changes in catecholamine content in the adrenal glands were found (table 1).

In control animals, with administration of physiologic

TABLE 1

Adrenaline, noradrenaline, and DOPA content in the adrenal glands of rats subjected to cooling and immobilization ($\mu\text{g/g}$)

Groups of Rats	No. Tests	Adrenaline	Noradrenaline	DOPA
Control	20	476 \pm 47	257 \pm 36	32.9 \pm 4.1
Subjected to cooling and immobilization	12	281 \pm 25.7*	155 \pm 45*	22.4 \pm 3.9*
Subjected to cooling	10	453 \pm 48	276 \pm 59	--
Subjected to immobilization	16	227 \pm 18*	430 \pm 55	23.7 \pm 4.0*

Note. Animals of all groups were examined on a background of physiologic saline solution administration: * = the difference is statistically reliable as compared to the control group

TABLE 2

Catecholamine content in the heart, hypothalamus, and blood with the influence of cooling and immobilization

Group of Rats	No. of tests	Heart ($\mu\text{g/g}$)		Hypothalamus ($\mu\text{g/g}$)		Blood ($\mu\text{g/l}$)	
		adrenaline	noradr.	adr.	noradr.	adr.	nor.
Control	12	0.069 \pm 0.009	0.39 \pm 0.06	0.25 \pm 0.04	1.74 \pm 0.3	5.0 \pm 0.5	8.5 \pm 0.9
Subjected to cooling and immobilization	12	0.054 \pm 0.007	0.36 \pm 0.04	0.27 \pm 0.07	1.6 \pm 0.22	4.2 \pm 0.45	9.3 \pm 0.95

Note. The rats were studied on a background of physiologic saline solution administration.

saline solution, no correlations between the adrenaline content in the various tissues was discovered. With the consecutive effects of cooling and immobilization, positive correlations were found between the adrenaline content in the blood and adrenal glands ($r = 0.56$), in the blood and hypothalamus ($r = 0.79$), in the blood and heart ($r = 0.57$), and in the adrenal glands and heart ($r = 0.72$).

The histochemical studies substantiated the data obtained in the biological investigation.

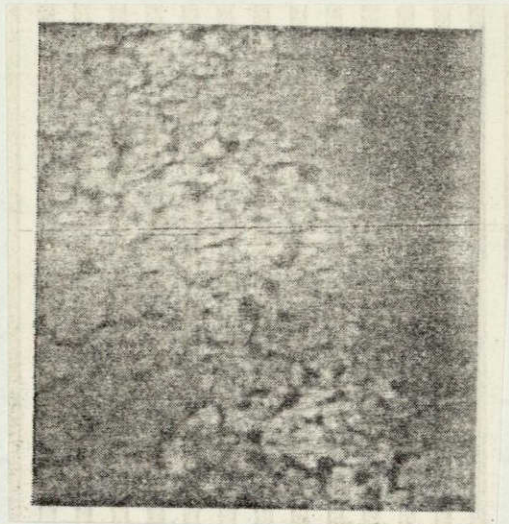
In control animals in the cerebral layer of the adrenal gland a yellowish green luminescence of high intensity is found (fig. 1). The luminescence is absent in the cortical layer of the adrenal gland. In the wall of the carotid artery, there is normally rather many adrenergic nerve fibers, accompanied by fat cells (fig. 2). In the cardiac muscle, an adrenergic network of nerve fibers appears connected to the vessels as well as to the muscle fibers of the heart.

The consecutive effect of cold and immobilization led to a significant decrease of catecholamines in the cerebral layer of the adrenal glands and in the walls of the carotid arteries. In the heart, no noticeable changes of adrenergic mediators were found.

Following double stress, only small areas of luminescent cells remained in the cerebral layer of the adrenal glands, located mostly along the edge (fig. 3). With a great increase in these regions, a decrease in the intensity of luminescence

as compared to normal was also noted.

Following stress, a noticeable decrease in luminescent nerve fibers is noted in the wall of the carotid artery (fig. 4).



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Fig. 1. Specific luminescence in the cerebral layer of the adrenal glands of rats under normal conditions.

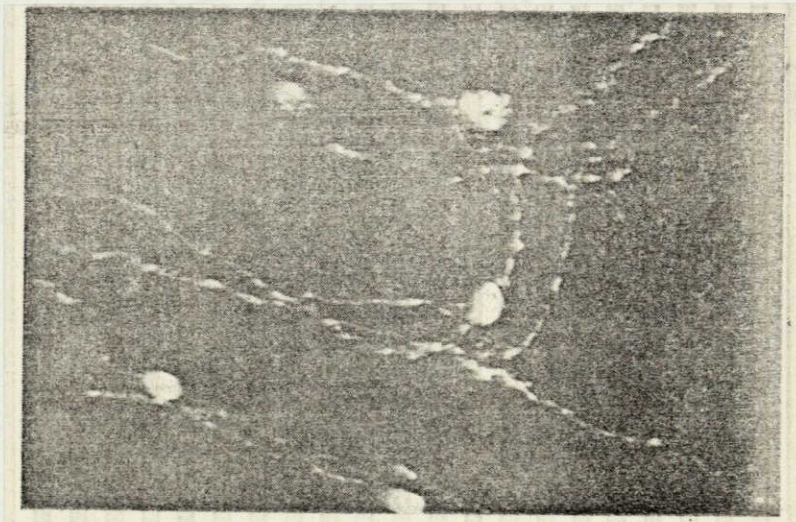


Fig. 2. Adrenergic nerve fibers and fat cells in the wall of the carotid artery of the same animal.

In experiments with the administration of 1-DOPA (3 times in 2 days at 45 mg/kg), an increase in the concentration of DOPA and adrenaline was noted in control rats in the adrenal glands (table 3). In rats subjected to the effects of cooling and immobilization, an increase in DOPA and noradrenaline content was found. The increase in adrenaline concentration was not statistically reliable. The total increase in catecholamines (adrenaline and noradrenaline) in control and test rats was about the same and corresponded to 1190 and 1151 μM respectively.

In experiments in vitro (table 4) it was shown that catecholamine synthesis in the adrenal glands of rats subjected to cooling and immobilization, in the presence of 1-tyrosine substrate, occurred more actively than in intact animals. These differences are due in a greater degree to noradrenaline than to adrenaline. In the heart, no difference was found in noradrenaline synthesis in intact rats and those subjected to stress.

DISCUSSION OF THE RESULTS

Study of the catecholamines contents in the tissue of rats following the consecutive effects of cooling and immobilization revealed a decrease in the adrenaline and noradrenaline content in the adrenal glands. Control experiments established that the lowering of the adrenaline content in the adrenal glands was linked to the effect of immobilization. Twenty four

hours after cooling, no statistically reliable changes were found in the adrenaline content of the rats' adrenal glands. The decrease in catecholamine content of the adrenal glands brought on by immobilization has been described previously¹².

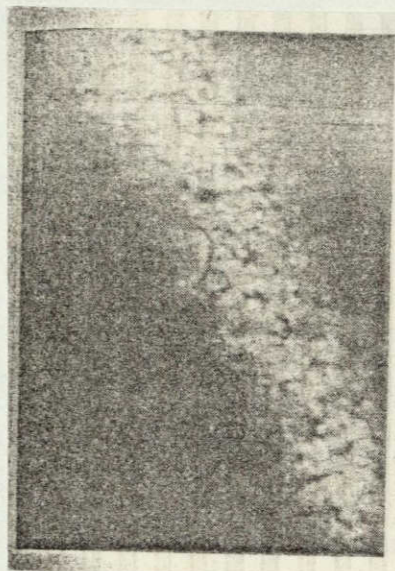


Fig. 3.

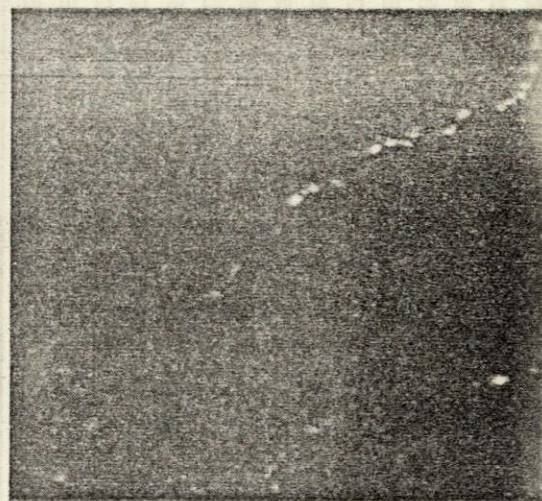


Fig. 4.

Fig. 3. Individual regions of luminescent cells in the cerebral layer of the adrenal glands of rats after the consecutive effects of cooling and immobilization.

Fig. 4. Adrenergic nerve fibers in the wall of the carotid artery of rats with the consecutive effects of cooling and immobilization.

As previously mentioned, with the consecutive effects of cooling and immobilization, there was a lowering not only of the adrenaline content, but of the noradrenaline content as well, while with immobilization alone, the noradrenaline content increased. Consequently, under the influence of the consecutive effects of both of these factors we notice not a

simple summation of the shifts due to each of these factors individually, but a more profound change occurs, leading to a decrease in concentration of both hormones of the adrenal glands' cerebral layer. At the same time it was shown that with the consecutive effects of cooling and immobilization in the heart, hypothalamus, and blood, no significant changes occurred in the concentration of the substances under study.

The combination of lowered catecholamine level in the adrenal glands with the absence of increased concentration of the hormones in the blood allows the shifts revealed to be attributed to the third phase of change in activity of the cerebral layer under conditions of stress^{2,4}.

TABLE 3

Influence of l-DOPA administration on catecholamine content in the adrenal glands of intact rats and those subjected to the consecutive effects of cooling and immobilization ($\mu\text{g/g}$)

Group No.	Group of rats	Solution administered	No. of tests	Adrenaline	Noradr.	DOPA
1	Control	Physiologic saline	20	476 \pm 47	257 \pm 36	32.9 \pm 4.1
2	Control	l-DOPA	12	639 \pm 34*	306 \pm 42	45.0 \pm 4.2*
3	Subjected to cooling and immobilization	Physiologic saline	12	286 \pm 26*	155 \pm 45*	22.4 \pm 3.9
4	Same	l-DOPA	12	343 \pm 42	290 \pm 55**	55 \pm 5.4**

Note. * = difference statistically reliable compared to group 1; ** = difference statistically reliable compared to group 3.

TABLE 4

Catecholamine synthesis in the adrenal glands and heart of intact rats and those subjected to cold and immobilization stress in the presence of l-tyrosine.

Group of Rats	Amount synthesized (in m M/g/hour)			in the heart noradrenaline
	in the adrenal glands adrenaline	noradrenaline	total	
Intact	340±17	260±24	600	4.75±0.48
Subjected to cooling and immobilization	430±30*	400±44*	830	5.9±0.51

Note. * = difference statistically reliable.

Analysis of the correlative relationships between catecholamines in various tissues of control animals and with the consecutive effects of cooling and immobilization allows us to confirm and deepen somewhat the relationships obtained by the computation and comparison of the arithmetic mean values. In control animals with administration of physiologic saline solution no reliable connections between the adrenaline contents in the various tissues were revealed. With consecutive cooling and immobilization a positive correlation between adrenaline content in the blood and adrenal glands was noted, which may be the result of increased synthetic capacity of the adrenal glands and release of adrenaline into the blood. Under conditions of stress^{2,4}, adrenaline penetrates more extensively into the hypothalamus and the cardiac tissue. This apparently explains the existence of a positive correlation

between adrenaline in the blood, on the one hand, and adrenaline in the heart and hypothalamus on the other. The positive correlation found between the heart and the adrenal glands is a result of the positive correlation blood — adrenal gland and blood — heart.

In the histochemical studies of the adrenal glands, data obtained by the biochemical method were substantiated, and also a decrease in the concentration of adrenergic mediators in vessel walls was shown.

In connection with the decrease in catecholamine content in the adrenal glands, it seemed advisable to investigate the reasons for the shifts revealed. The assumption is improbable that the decreased catecholamine concentration in the adrenal glands results from increased catecholamine secretion, since the catecholamine content in the blood in our experiments did not increase. The assumption was made that decreased adrenaline and noradrenaline content in the adrenal glands may be due to a decrease in their biosynthesis or to a decreased supply of substrates for catecholamine synthesis. To verify one of these assumptions in the subsequent series of experiments, an attempt was made to evaluate the synthetic capacity of the adrenal glands. It was shown that in the presence of the substrate l-tyrosine, catecholamine synthesis in the adrenal glands of rats subjected to cooling and immobilization occurs more actively than in intact animals. These data

agree with the results of studies in which it was determined that with cooling and immobilization, the rate of catecholamine metabolism in the adrenal glands increases and tyrosinehydroxylase activity is raised⁹⁻¹¹.

In studying the synthetic capacity of the adrenal glands of intact animals in a separate series of experiments, aside from adrenaline and noradrenaline synthesis, the formation of dopamine and DOPA were also investigated and it was shown thereby that the biosynthesis of dopamine and DOPA does not surpass 2 % of adrenaline and noradrenaline synthesis³. Consequently, the sum of the adrenaline and noradrenaline formed practically corresponds to the amount of tyrosine subjected to the effect of tyrosinehydroxylase and their total content may to a certain extent serve as a measure of this enzyme's activity. It is interesting to note that according to our data the tyrosinehydroxylase activity, calculated by the suggested method, is in the intact animal 600 m μ M/g/hour, while according to the data of Nagatsu and Yamamoto¹³, authors of the method for determining tyrosinehydroxylase activity, it is 431 m μ M/g/hour, i.e. the activity of the enzyme studied by us is in any case no lower than the results determined by other authors. With the consecutive effects of cooling and immobilization, tyrosinehydroxylase activity increased, according to our data, and was equal to 830 m μ M/g/hour. The increased capacity of the adrenal glands to synthesize catecholamines revealed in our study and the increased

tyrosinehydroxylase activity allowed us to draw the conclusion that the decreased catecholamine concentration in the adrenal glands, induced by consecutive cooling and immobilization, is not due to depression of the synthetic processes, is not dependent on increased secretion of the hormone (there is no increase in adrenaline content in the blood), and it is possibly due to a lack of substrates appearing in the adrenal glands and permitting synthesis of the catecholamines.

The results obtained served as a basis for carrying out experiments with administration of the catecholamines' precursor: l-DOPA. In this, preference was given l-DOPA and not l-tyrosine, since for the effects of the latter to appear a longer period of time is required. Further, in order to strengthen the effect of the l-DOPA, we tried administration of it 3 times instead of once, described in detail in our studies and in the works of other authors^{1,5,6,8,14}.

It was shown that in both control rats and in those subjected to cooling and immobilization, rats under the influence of l-DOPA showed approximately the same total accumulation of catecholamines. However, in test rats the accumulation was primarily noradrenaline, while in control rats it was adrenaline. It is possible that the differences are due to decreased phenylethanolamine-N-methyltransferase activity.

The results obtained allowed us to conclude that

administration of l-DOPA to rats subjected to cooling and immobilization, as well as under the influence of pain, under the influence of trauma, with neurological degeneration, or radiation exposure^{1,5,8,14} may lead to a restoration of the low catecholamine level in tissues.

CONCLUSIONS

1. The results of the biochemical and histochemical studies showed that with the consecutive effects of cooling and immobilization a decrease in the adrenaline and noradrenaline content in the adrenal glands is noted. At the same time, a simple summation of the changes induced by the individual effect of cooling and immobilization is not found, but rather a more profound change occurs.

2. Study of the synthetic capacity of the adrenal glands in the presence of the substrate l-tyrosine showed that with the consecutive effects of cooling and immobilization, an activation of catecholamine synthesis is observed.

3. Administration of l-DOPA (3 times in 48 hours at 45 mg/kg) induced activation of catecholamine synthesis in the adrenal glands of control and test animals. Consequently, administration of l-DOPA prevents change in the catecholamine content of the adrenal glands caused by stress agents.

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